Use of Acute and Chronic Bioassays to Assess the Applicability of Selected Advanced Wastewater Treatment Technologies for the Green Bay Metropolitan Sewerage District

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Several state-of-the-art advanced wastewater treatment technologies were evaluated during pilot studies. All treatment endpoints received extensive chemical analysis as well as whole effluent bioassays. Results indicated that effluent from the existing carbonaceous treatment is toxic most of the time, whereas nitrified effluent streams showed no acute or chronic bioassay failures. Subtle effects on Ceriodaphnia were, however, observed. Tertiary treatment typically reduced these effects, though one treatment system introduced another source of toxicity inherent to the chemical process. Interpretation of bioassay results were further complicated by inconsistencies within the chronic test statistics procedure. These observations support the need to review all data generated during bioassays, such as mean growth rates or reproduction, rather than chronic "pass/fail" endpoints alone.

Introduction
The Green Bay Metropolitan Sewerage District (GBMSD) provides wastewater treatment for nine communities and two large pulp and paper industries. The existing facilities were placed into operation in 1975 and were designed to meet the community's needs through the year 1990.

The District's operation has consistently maintained compliance with EPA categorical effluent limits of 30 mg/l BOD and TSS, and a 1.0 mg/l total phosphorus limit as established by an International Joint Commission (IJC) Agreement between the U.S. and Canada.

The treatment plant effluent discharges to the Fox River at the mouth of Green Bay, Lake Michigan. Historical water quality problems of the Fox River and lower Green Bay have been well documented (Bertrand et al. 1976; Day 1978; Howmiller and Beeton 1971; Patterson et al. 1975; Peterman et al. 1980; Smith et al. 1988; Sullivan, and Delfino 1982). The lower Fox River/ Green Bay area has been designated as one of the 42 "Areas of Concern" by the IJC.

In 1987 the Wisconsin Department of Natural Resources (WDNR) issued notice of its intent to reissue a Wisconsin Pollutant Discharge Elimination System (WPDES) discharge permit to the GBMSD. The permit was to contain new and more stringent limits for CBOD, chlorine residual, fecal coliform and whole effluent toxicity, along with recommendations pertaining to future monitoring and effluent limits for certain toxic compounds, including ammonia, heavy metals and residual organics.

The Wisconsin DNR has recently developed new administrative codes NR105 and 106 for the control of toxics from point sources. These codes address over 100 toxic compounds, and also enable the WDNR to place a bioassay effluent limit or monitoring requirement in a WPDES permit.

Existing effluent data indicated the possibility of noncompliance with future permit conditions. Whole effluent bioassays performed in 1987 showed both acute and chronic failure to fathead minnows and Ceriodaphnia (Buttke and Rades 1987a,b).

GBMSD therefore commissioned a facility plan to address these and other issues. A major component of the plan included extensive pilot studies using state-of-the-art Advanced Wastewater

Treatment (AWT) technologies. The pilot studies were designed to evaluate alternative AWT processes likely to ensure compliance with the proposed and potential future permit requirements.

Methodology

Pilot studies were conducted in 1987 and 1988, with the majority of work occurring between November, 1987 and March, 1988. Processes investigated include:

- Single-stage nitrification (identified as B12)
- Powdered Activated Carbon (PACT™) nitrification
- Alum/sulfide treatment
- High-lime treatment
- Filtration
- Carbon adsorption

Four pilot study tests evaluated single-stage nitrification followed by the AWT systems. Four more tests evaluated the PACT™ process followed by the AWT systems.

Each location within the pilot system identified as a possible treatment endpoint was sampled intensively for chemical parameters and whole effluent bioassays, including: activated sludge nitrified effluent (B12); B12 after chlorination/dechlorination; PACT™ secondary effluent; PACT™ filter effluent; alum/sodium sulfide filter effluent; alum/sodium sulfide carbon column effluent; high lime recarbonation clarifier effluent; high lime filter effluent; high lime carbon column effluent; and existing carbonaceous effluent (B15). A total of eight 7-day bioassays were performed on eight effluents and a Green Bay dilution water control. All tests were conducted in strict accordance with EPA protocol (Horning and Weber 1985). The acute bioassay measures percent survival in 100% effluent. The chronic bioassay determines any sublethal effects, measured as reduction in growth for fathead minnows, or a decrease in Ceriodaphnia reproduction. The chronic "pass/fail" endpoint is based on observed sublethal effects at a particular effluent concentration, termed the "Instream Waste Concentration" (IWC). This concentration is defined as the percentage composition of the effluent in the receiving water stream assuming a stream flow of 25% of the historical minimum 7-day flow expected once in ten years (7-day Q_{10}). The chronic test statistics calculate a "No Observed Effect Concentration", or N.O.E.C. The GBMSD IWC was calculated to be 34%. Therefore, the N.O.E.C. calculated from a given test must be at least 34% to pass the chronic criterion.

Results and Discussion

A total of eight 7-day test periods were utilized for bioassay analysis. Four of these included full-scale nitrification as the effluent source to the tertiary systems, while four runs reflect use of the PACT™ pilot plant effluent to drive the tertiary systems. Each 7-day run is identified by the "mode" of nitrification, e.g. "B12" or "PACT™", followed by the chronological run number.

Table 1 presents an overall summary of the 63 total acute and chronic bioassays performed. A total of six acute failures were noted; five in the existing carbonaceous effluent (B14/15), and one in the alum filter effluent. The B14/15 mortalities were presumably due to high ammonia levels, while residual sulfide was believed to be responsible for the alum mortality.

Referring to Table 1, a total of 12 chronic failures were noted during the entire study. Six of these corresponded to the B14/15 effluent. Of the remaining six failures, three were in the alum filter, two in the lime filter effluent, and one in the

Table 1. Number of acute and chronic bioassay failures observed during the GBMSD Pilot Study.

Effluent	Acute Bioassay Results				Chronic Bioassay Results			
	Fathead Minnow		Ceriodaphnia		Fathead Minnow		Ceriodaphnia	
	N	Failures	N	Failures	N	Failures	N	Failures
Full Scale Nitrification								
B12	4	0	4	0	3	0	4	0
B12 (chlor/dechlor)	2	0	2	0	2	0	2	1
Alum Filter Eff.	4	0	4	1	3	0	4	0
Alum CC Eff.	4	0	4	0	3	0	4	0
Lime Recarb Eff.	4	0	4	0	3	0	5	0
Lime Filter Eff.	4	0	4	0	3	1	3	1
Lime CC Eff.	4	0	4	0	3	0	4	0
PACT™ Nitrification								
PACT™ Secondary Eff.	4	0	4	0	4	0	4	0
PACT™ Filter Eff.	4	0	4	0	4	0	4	0
Alum Filter Eff.	4	0	4	0	4	0	4	3
Alum CC Eff.	4	0	4	0	4	0	4	0
Lime Recarb Eff.	4	0	4	0	4	0	6	0
Lime Filter Eff.	4	0	4	0	4	0	2	0
Lime CC Eff.	4	0	4	0	4	0	4	0
Carbonaceous								
B14/15	8	5	8	0	7	5	8	

B12-1 (chlorination/dechlorination) effluent. Figure 1 presents a graphical depiction of chronic bioassay results for run B12-3. Results are displayed for both fathead minnow and Ceriodaphnia. All graphs contain results from the 100% effluent analyses only. Each summary graph for fathead minnow data includes percent survival (bar graphs represent actual percent survival) and mean final weights including 95% confidence interval. N.O.E.C. values, as calculated by WDNR computer programs, are listed in parentheses above each bar or mean weight interval. Results for Ceriodaphnia include percent survival (again shown by bar graphs) and mean number of offspring per individual including 95% confidence interval. N.O.E.C. values are also listed above each bar or mean number interval. The following paragraphs discuss bioassay results in more detail, grouped by treatment system.

Carbonaceous Effluent (B14/15)

Significant detrimental effects were noted on all B14/15 bioassays. For fathead minnows, five of seven chronic tests yielded failures. One out of eight Ceriodaphnia tests failed. As previously noted, ammonia is thought to be the main source of toxicity. Fathead minnows are known to be highly

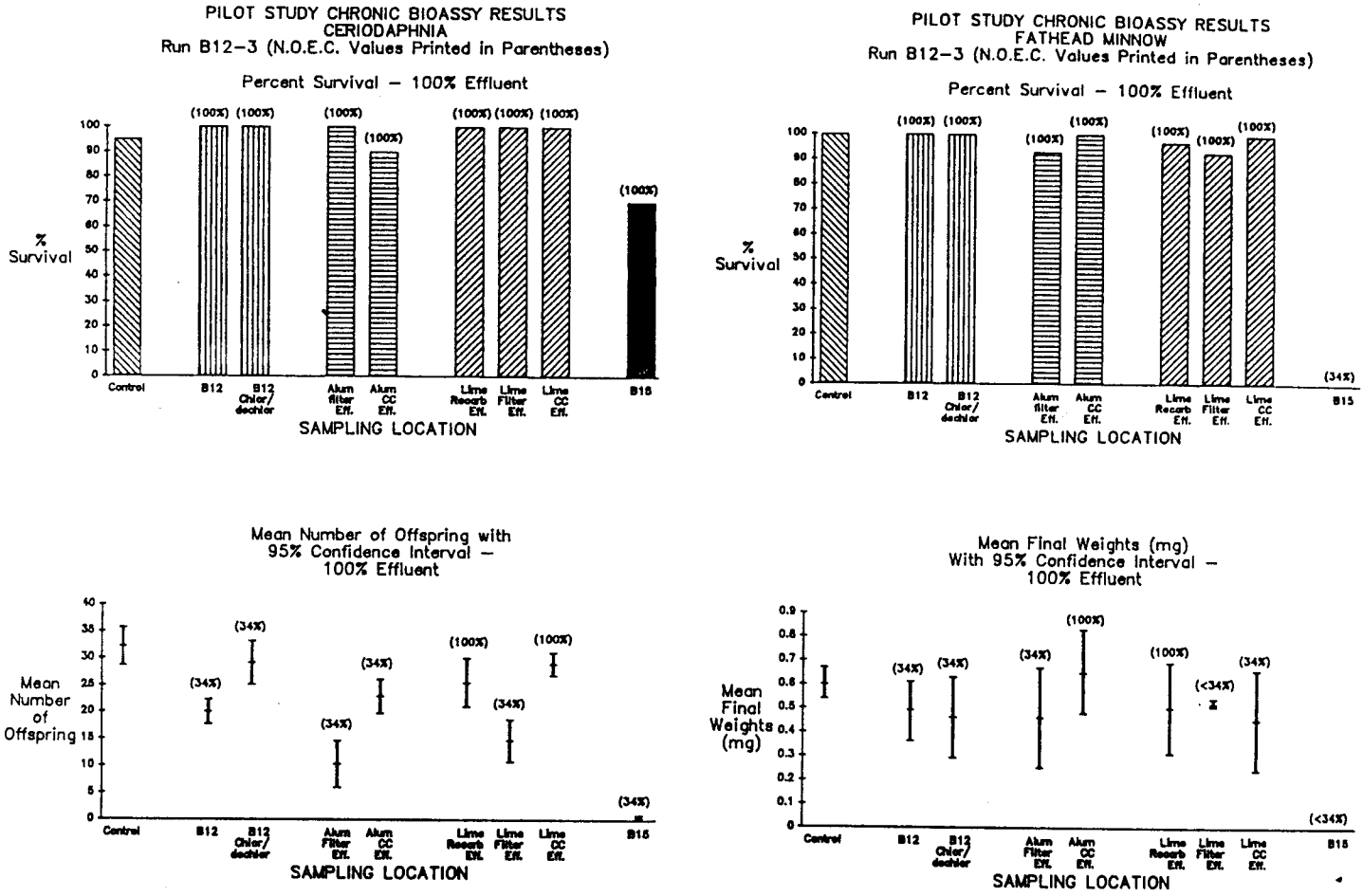

Figure 1. GBMSD pilot study bioassay results, run B12-3.

sensitive to ammonia, and this is reflected in the test results.

Full Scale Nitrification

Full scale nitrification yielded an effluent which passed all bioassays during the test period. Ammonia levels were generally low (less than 1.0 mg/l), further supporting the proposition that ammonia is the primary toxicant in the GBMSD effluent.

Fathead minnow results for all four B12 runs showed no significant difference to the control (all N.O.E.C. values 34% or greater). Some effect in Ceriodaphnia reproduction was noted in runs B12-1, B12-3 and B12-4, even though all N.O.E.C. values were 34% or above.

The WDNR had expressed interest in including bioassay analysis of a chlorinated/dechlorinated effluent to determine whether this process might in itself cause a toxicity problem. Therefore, simulation of chlorination/dechlorination was performed in the laboratory on B12 effluent during runs B12-2 and B12-3. Samples were first chlorinated at an approximate dosage of 10 mg/l for 30 minutes, then dechlorinated with sodium thiosulfate. These samples were then used for bioassay analysis. Fathead minnow results were identical to unchlorinated B12 effluent, but Ceriodaphnia reproduction was significantly reduced during run B12-2. Therefore, the dechlorination procedure was modified so that concentrations of excess sodium thiosulfate were minimized.

Samples from run B12-3 showed identical Ceriodaphnia results to unchlorinated B12 effluent.

PACT™ Nitrification
Bioassays were performed on two effluents from the Zimpro PACT™ pilot plant: secondary effluent; and, filter effluent. No chronic failures were noted throughout the four PACT™ runs. Fathead minnow results were very similar to the control. Ceriodaphnia reproduction was slightly effected during runs PACT™-3 and PACT™-4.

Alum AWT
Bioassays were performed on two effluents from the alum AWT system: alum filter effluent; and, alum carbon column effluent.

Fathead minnow results showed no significant difference from the control on all eight runs except for run B12-3, when the filter effluent showed a slight effect (N.O.E.C. of 34%).

Ceriodaphnia results, however, showed significant toxicity-related effects. Recall that run B12-1 showed an acute toxicity failure for Ceriodaphnia in alum filter effluent. This problem was believed to be related to residual sulfide from the alum/sodium sulfide treatment, in conjunction with the short detention time of the pilot system. A secondary aeration/holding tank was incorporated into the system hoping to drive off any residual sulfide, and no more acute failures were observed. However, chronic effects were noted throughout the remaining bioassays. Three of the eight alum filter effluent Ceriodaphnia bioassays failed the reproduction test. However, even the five tests which passed showed obvious detrimental effects. It was further noted that during four out of the eight runs, the carbon column treatment step improved conditions to the point that the bioassay results were not significantly different from the control. The remaining four runs showed improvement, but to a lesser degree.

It was thought, at first, that the added aeration step had alleviated the sulfide problem, as the next few bioassays yielded no failures. It later became apparent that a toxicity problem in the alum system was still present. In order to verify the effectiveness of the aeration tank, five grab samples of filter effluent were collected during run PACT™-4. Results showed a range of 41 to 74 µg/l (ppb) residual sulfide. One sample was split prior to analysis, with one aliquot receiving an extra hour of vigorous aeration in the laboratory prior to analysis. This extra aeration step reduced the residual sulfide level from 41 to 35 µg/l.

Residual sulfide levels at these concentrations could be the source of toxicity in the alum system. The EPA "Gold" book criterion for undissociated H_2S for fish and aquatic life (in fresh and marine water) is 2.0 µg/l. Residual sulfide levels found in the alum system, however, are not identical in form to undissociated H_2S. Sulfide exists in three forms in water; H_2S, hydrosulfide (HS^-) ions or sulfide ($S^=$) ions. The proportion of each is controlled primarily by pH. As pH drops below 9.0, the proportion of undissociated H_2S (and therefore the toxicity) increases. The aeration step which was added to the alum pilot system increased the alum filter pH from approximately 7.1 to 8.0. This aeration-induced pH increase may have served to reduce the sulfide toxicity, rather than reducing the sulfide concentration.

To further investigate the sulfide toxicity problem, the pilot system was operated again in May, 1988. Ceriodaphnia bioassays were performed on alum filter effluent, both before and after a chlorination/dechlorination procedure. The chlorination process was suggested as a possible means of oxidizing any residual sulfide. The chlorination/dechlorination procedure

was identical to that which was used on earlier bioassays, using sodium thiosulfate to dechlorinate.

Results of sulfide analyses indicated that the chlorination process did remove approximately half of the residual sulfide, though daily variability was considerable. Sulfide levels after chlorination ranged from <2 µg/l to 78 µg/l. Bioassay results were very similar to the previous eight tests, showing significant effect on Ceriodaphnia reproduction. The chlorination/dechlorination process reduced the level of toxicity, but to only a minor degree.

High Lime AWT

Bioassays were performed on three effluents from the high lime AWT system: recarb clarifier effluent; lime filter effluent; and, carbon column effluent.

Fathead minnow results showed no significant difference from the control on five of the eight (total) runs. Run B12-2 showed a significant effect for all three effluents, presumably caused by very low (approximately 20 mg/l) alkalinity concentrations observed in the lime system during this run. Lime system alkalinity values measured during the other pilot runs were all above 30 mg/l. It is thought that the lower than average operating load from one of the two paper mill influent streams is the reason for the low alkalinities seen during run B12-2. Alkalinity values close to 20 mg/l have the effect of increasing the toxicity of heavy metals and other compounds. It is believed that the B12-2 run results reflect this phenomenon.

In order to prove that the observed toxicity was alkalinity related, a duplicate series of lime system effluents with added $NaHCO_3$ were tested during the next bioassay. However, alkalinities in the lime system returned to the 30-40 mg/l range, and no toxicity was observed in either sample series.

The high lime system consumes alkalinity during the chemical reactions involved during treatment. Even though the other seven bioassays showed no repeat of this occurrence, it should be considered a potential problem for future full-scale application.

The lime filter effluent sample failed the fathead minnow growth test on run B12-3 (shown on Figure 1 as <34%). However, it appears that this failure is more related to a statistical error than to toxicity. The confidence interval around the mean weight value is extremely small, normally an indication of high data reliability. This narrow range of variability, however, allows the WDNR statistics program to detect very small differences when compared to the control. In effect, if the replicate weight variations had been greater, the N.O.E.C. would have been much higher, even if the mean value remained the same. Realistically, therefore, this test should not be considered a "fail".

An unusual event occurred with the fathead minnow bioassays during run PACT™-4. Relatively high mortalities were observed in the lime system samples for one day of the test, corresponding to effluent collected on February 18, 1988. The number of mortalities decreased as treatment advanced (i.e. most mortalities in recarb clarifier effluent, least in carbon column effluent). No further mortalities were observed. Currently, there are no obvious explanations for this occurrence. Review of chemical data shows no obvious problems, and no operational difficulties were noted. Even so, no acute or chronic test failures were observed for the run.

Ceriodaphnia results from high lime system bioassays indicate a subtle recurring effect on reproduction, particularly in the lime filter

Table 2. Ammonia concentrations measured in bioassay samples during the GBMSD Pilot Study. (Weekly average value followed by daily range.)

	Ammonia-Nitrogen (mg/l)	
Run	**B14/15**	**B12**
B12-1	17	<1
B12-2	13	<1
B12-3	22	2.3 (<1-5.4)
B12-4	14	<1
	B14/15	**PACT™**
PACT™-1	16	1.8 (<1-5.6)
PACT™-2	13	3.0 (<1-12.1)
PACT™-3	18	<1
PACT™-4	14	1.1

effluent. There were no chronic Ceriodaphnia failures observed in seven out of eight runs, but often times the N.O.E.C. values decreased through the system, an obviously anomaly. Lime filter effluent failed the reproduction endpoint on run B12-2, but it appears the previously discussed concerns regarding statistical evaluation of a narrow confidence interval may again be the reason for the failure.

An alteration to the Ceriodaphnia bioassay regime was made during the last three runs: PACT™-3, PACT™-4 and B12-4. This change entailed collecting a lime clarifier effluent sample (normally at pH = 11.2) and using CO_2 gas to adjust the pH to 7.0 prior to use in a bioassay. (The pilot system normally used sulfuric acid to adjust pH.) Therefore, Ceriodaphnia bioassays were performed on recarb clarifier effluent, lime clarifier effluent W/CO_2, and lime carbon column effluent during these runs. The recarb clarifier and lime clarifier W/CO_2 samples should have been identical except for the method of pH adjustment.

Results from this assessment were inconclusive. For runs B12-4 and PACT™-3, the two effluents had identical results. For run PACT™-4, the N.O.E.C. value for clarifier effluent W/CO_2 was lower than the recarb clarifier effluent value.

It appears, therefore, that some system-related chemical reaction or other factor may be exerting a small but measurable effect on the Ceriodaphnia chronic bioassay.

Ammonia Concerns

Historical bioassay results had indicated that ammonia was thought to be a major source of toxicity observed in GBMSD effluent. The GBMSD Facilities Plan intends to address ammonia, and so it was hoped to perform all pilot study bioassays on ammonia-free effluent in order to identify any other toxicity causing compounds. However, due to fire-related operational problems at one of the paper mills, influent ammonia concentrations sometimes exceeded nitrification capacities resulting in ammonia bleed-through to the tertiary treatment systems.

Table 2 presents ammonia concentrations throughout the pilot system during the study. Ammonia values exceeded 1.0 mg/l (weekly average) on three out of the eight runs. The highest daily value was reported

during run PACT™-2, at 12.1 mg/l. The weekly average for this run was 3.0 mg/l. Bioassay results from PACT™-2 and B12-3 indicate a slight effect noted for fathead minnow growth, though no test failures occurred.

Weekly average ammonia values for existing carbonaceous effluent (B14/15) are also included in Table 2. It is interesting to note that for an average ammonia concentration of approximately 16 mg/l (entire study), failure rates for acute and chronic bioassays were 63% and 71%, respectively.

Bioassay Procedure Concerns

The 7-day chronic bioassay test procedures, as conducted during the GBMSD pilot study, have been developed primarily by the EPA. Several changes in techniques and procedures have been made during recent years, and even today, the methods appear to be in a state of continuing evolution.

The GBMSD experience with the test methods, themselves, was mostly positive. Overall, the tests appear to be credible and repeatable. It is interesting to note that the two organisms seem to respond quite differently to differing toxic agents, thus supporting their selection as complementary test animals.

Bioassay results have identified possible toxicity problems affiliated with some of the treatment systems tested, even when results of chemical analysis do not clearly show such evidence. However, during review of multiple data sets, several inconsistencies were noted relating to the statistical program which calculates final N.O.E.C. values.

For example, Green Bay dilution water used during the first three runs of the pilot study caused significant mortality to fathead minnows, both in the control samples themselves and in the 34% effluent samples. The problem appeared to be excessive numbers of bacteria or fungi in the bay water. Fish that died were observed to have fungus-like growths in their gut, and a mat-like layer developed on the bottom of the 34% effluent beakers each day. (This problem was eliminated by changing the water collection site from the east shore to the west shore.) The statistics program responded to this condition by lowering the "standards" of the test, in one case assigning a N.O.E.C. value of 100% to an effluent that achieved only 57% survival in 100% effluent. A later test, with 100% control survival, calculated a N.O.E.C. value of 34% for an effluent that achieved 87% survival in 100% effluent. Therefore, it appears that it is to the dischargers advantage to conduct effluent bioassays using dilution water that is mildly toxic. Clearly, improvements in the statistical analysis program would seem appropriate.

Another inconsistency involves the previously discussed situation where replicate variability is very low, allowing the statistics to detect very small differences between mean values of the control and the effluent. This means that the statistics seem to expect variability between replicates, and that a high degree of confidence regarding the actual test data may actually result in a lowered N.O.E.C. value. It would seem prudent, therefore, to review all bioassay results, such as that included in Figure 1, rather than to judge the test based strictly on N.O.E.C. values.

The GBMSD experience regarding EPA/WDNR bioassay test procedures was acceptable, though some inconsistencies with the statistics program were noted. Our experience tends to support the need to review bioassay results from a biological as well as a mathematical perspective. Bioassay data generated were useful in the final selection process within the GBMSD Facilities Plan. Figure 2 contains a comparison of bioassay results between existing carbonaceous effluent

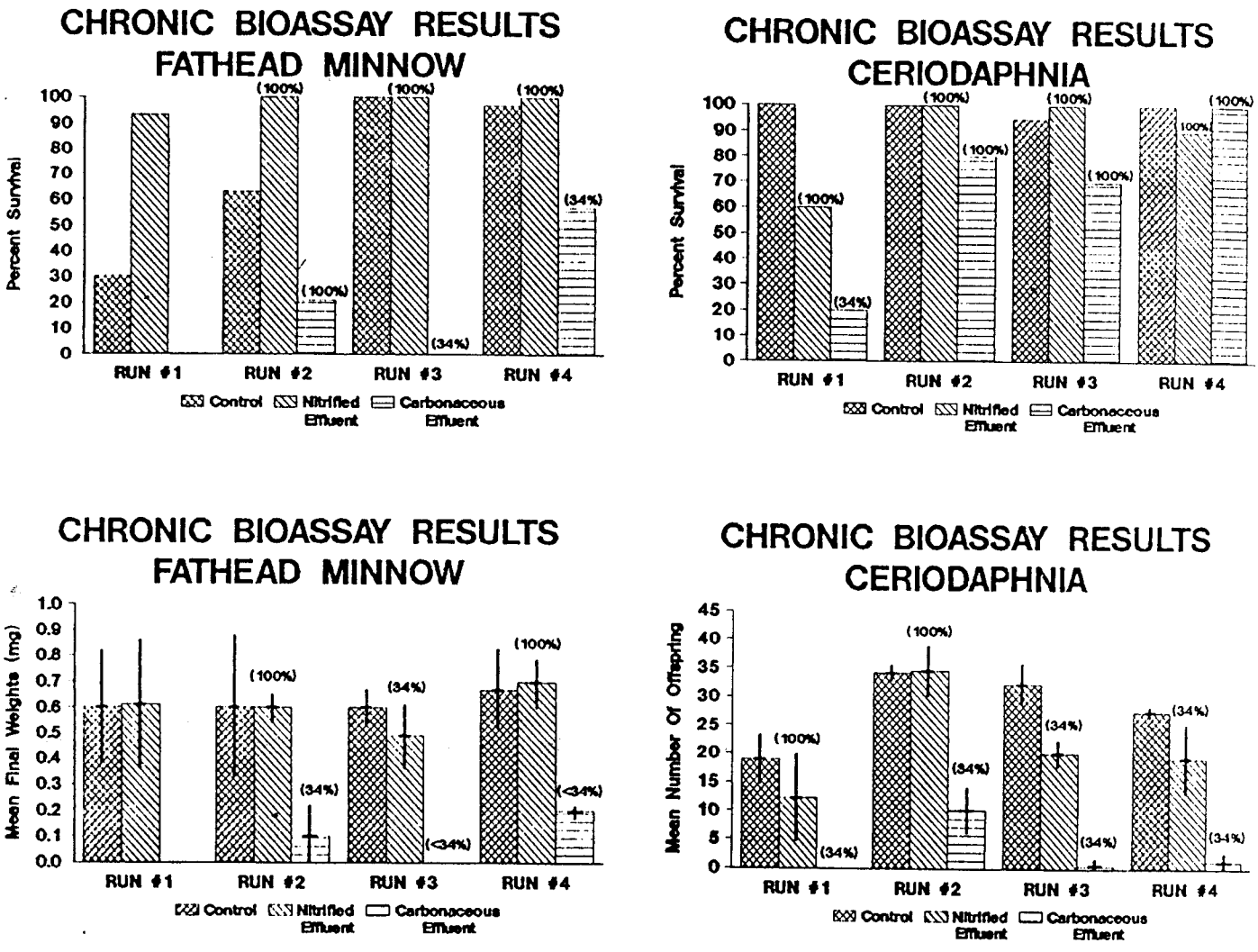

Figure 2. Comparison of bioassay results between GBMSD carbonaceous effluent, nitrified effluent, and Green Bay control water.

and full scale nitrification. Results from bioassays performed on nitrified effluent show a significant improvement over carbonaceous effluent. In fact, nitrified effluent results show only minimal variation from the receiving water controls.

Summary

A total of eight 7-day chronic bioassays were performed on eight effluents during the GBMSD pilot study. Excluding existing carbonaceous effluent (B14/15), only one acute and six chronic failures were observed throughout the entire test period.

Effluent from the full scale nitrification quadrant (B12) passed all acute and chronic bioassays. Fathead minnow results from all runs showed no significant difference from the control. A slight effect was noted in Ceriodaphnia reproduction, though not to the level of test failure.

Results from the PACT™ pilot plant effluent were very similar to B12 effluent with no apparent effect noted on fathead minnows, but a slight

effect noted in Ceriodaphnia reproduction.

Results of the bioassay program show concern regarding residual sulfide levels in the alum/sodium sulfide treatment system. Significant reductions in Ceriodaphnia reproduction were noted, including one acute and three chronic failures. Subsequent testing indicated that residual sulfide is a definite problem with this form of treatment, though it is not known how the pilot scale results would apply to a full-scale operation.

Three separate effluents of the high lime system were analyzed. The effluent from this treatment system is characteristically low in alkalinity. Bioassay results have shown that the effluent alkalinity must be maintained at 30 mg/l or more in order to minimize increased toxicity from various compounds. A slight reduction in Ceriodaphnia reproduction was noted in the lime system effluents and may be related to the system itself. Bioassays on effluent using CO_2 gas for pH adjustment, instead of sulfuric acid, showed no apparent improvement.

High lime system results are difficult to assess completely, as the nitrified effluent which fed the lime system was already relatively nontoxic. However, if bioassay results from the influentto the lime system showed subtle effects as compared to the controls, the lime treatment typically improved the results. As with the alum system, the carbon column polishing step significantly improved the Ceriodaphnia bioassay test results if the influent stream showed depressed reproduction.

Several inconsistencies were noted relating to the statistical program which calculates final N.O.E.C. values. Results obtained during the GBMSD pilot study support the need to review all bioassay results, such as graphical plots of actual data, rather than to judge the test based strictly on N.O.E.C. values.

Acknowledgements

Credits

The Institute of Paper Chemistry, Appleton, Wisconsin, was contracted to conduct the bioassay analyses. George Buttke served as Project Officer, while Dave Rades served as Project Administrator. The pilot study was a joint effort involving all divisions with the GBMSD. CH_2M Hill was the Facilities Plan consultant. This paper was accepted for presentation at the 62nd Annual WPCF Conference in San Francisco, but was cancelled due to the earthquake of October 17, 1989.

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